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Contents

ORIGINAL ARTICLES

PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

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ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

Under the above caption will be published from time to time comments, criticisms and suggestions on technical procedures; minor contributions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

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ERRATUM

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Under the heading "Calibration of Photelometer" in the article "An Improved Method for the Determination of Serum Bilirubin" the following corrections should be made:

Step 1. Stock Solution of Pure Bilirubin—Weigh 10 mgms. of pure bilirubin accurately and dissolve in 100 cc. of chloroform.

Step 3. Solution No. 2. Dilute 20 cc. of stock solution to 100 cc. with 95 per cent ethyl alcohol.

TOXICOLOGY IN A GENERAL HOSPITAL

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There has been an increasing tendency within recent years to neglect or, at most, to give very little consideration to toxicology and toxicological procedures in general hospitals. This is not surprising when one realizes that in many medical schools the subject usually is poorly taught or altogether neglected. The result of this poor training is reflected in the hospitals where the internes and younger members of the staff are helpless in the face of even quite simple toxicological problems.

A knowledge of some of the more simple procedures is obtained very easily and is quite valuable to any physician. Every hospital laboratory should be equipped and in position to do any of the procedures which shall be described. In this article an endeavor will be made to outline and bring together some of the more useful tests and to indicate in a general way their value in a general hospital. All the described tests and procedures are in use at the Peter Bent Brigham Hospital and have been selected from various sources in an endeavor to obtain as simple procedures as possible and still give the desired results. Case reports are given after certain procedures in order to illustrate their value. All case reports are from patients admitted to the Medical Wards of the Peter Bent Brigham Hospital.

ALCOHOL

Occasions frequently arise when an accurate estimation of the blood alcohol content would be of much value in determining a definite diagnosis. This is especially true in a busy out-patient

department where emergency cases arriving in coma with little or no history, signs of trauma and an odor of alcohol on the breath present a perplexing situation. How much simpler it would be if a sample of blood could be taken and within an hour's time the exact status of the patient's condition in regard to alcoholic narcosis determined. It might also be that occasionally a patient will wish to prove to the police that he is not intoxicated, even though he may have been involved in an automobile accident and a definite odor of alcohol can be detected on his breath from a previous cocktail.

Procedures for the determination of alcohol in quantitative amounts have been in use for many years. The more useful ones usually are based on some modification of the original procedure of Nicloux.[1] The one described here is that used by Fleming and Stotz[2] in their studies on the alcohol content of the blood and cerebrospinal fluid after a standard test dose. There are other procedures which are equally good, such as the one used by Miles[3] and the recently described method of Gettler[4], but this one has given quite good results in our hands and can be adapted readily to the photocolorimeter. The procedure is as follows:

1. Withdraw 5.0 cc. of the patient's blood into an oxalated tube. The blood may be placed in the icebox for 24 to 48 hours, if necessary, without any loss of alcohol.

2. Hemolyze 2.0 cc. of the blood by adding it to 14.0 cc. of distilled water in a 120 cc. Erlenmeyer flask.

3. Add to the hemolyzed blood 2.0 cc. of 10 per cent sodium tungstate and mix well.

4. Then add 2.0 cc. of ⅔ normal sulphuric acid, shake well, and let stand for 5 minutes.

5. Filter and pipette 10.0 cc. of the filtrate into a small round-bottomed distilling flash of 100 cc. capacity.

6. Connect the flask to a glass tube 8 mm. in diameter and 38 cm. long bent so that the short end of the U is connected to the distilling flask and the long end extends nearly to the bottom of the collecting cylinder.

7. Using a microburner, 6.0 cc. are distilled off in a 5 minute period.

8. The distillate is collected in a 25.0 cc. graduated ground glass stoppered cylinder which contains 5.0 cc. of 18 normal sulphuric acid and 2.0 cc. of standard potassium bichromate. During distillation the cylinder must be packed in cracked ice.

9. When distillation is complete, wash the tip of the tube with distilled water, the washings going into the cylinder. The cylinder contents should now be about 15.0 cc.

10. Dip the glass stopper of the cylinder into concentrated sulphuric acid which will seal it into the cylinder and place the cylinder in a water bath kept at 85° centigrade for 45 minutes. Be sure that the water of the bath is above the level of the contents of the cylinder.

11. Remove and cool. At this point the amount may be read directly on the photocolorimeter. If there is less than 1.0 mgm. of alcohol present in the cylinder, the color of the solution will be a faint yellow.

12. Add to the cylinder content either 5.0 or 7.0 cc. of standard ferrous ammonium sulphate, depending on whether there is much or little dichromate remaining. Be sure that a light bluish green color appears after adding the ferrous ammonium sulphate. If any yellow color remains, more ferrous ammonium sulphate must be added.

13. Mix well and titrate the cylinder contents with the standard potassium permanganate until there is an appearance in the cylinder of a faint pink color which remains after shaking. A microburette graduated in .02 cubic centimeters is used for adding the potassium permanganate.

Calculation:

$$50 \times \text{cc. of } K_2Cr_2O_7 - \left(\frac{\text{cc. of } FeSO_4\text{-cc. of } KMnO_4}{3}\right) = \text{mgm. of alcohol per}$$

100 cc. of blood. Normally with this method about 2 to 4 mgm. of alcohol will be found. Jungmichel[5] gives a good discussion of the relationship between blood alcohol content and the physical state of the patient. Any patient with a content over 300 to 500 mgm. usually is "dead drunk" and is quite likely to be fatally poisoned. The method can be used for estimation of the cerebrospinal fluid alcohol content, in which cases 1.0 cc. of the cerebrospinal fluid is taken and likewise one half of the precipitants and distilled water, but distillation is carried out on a 10.0 cc. sample as under blood.

The reagents required are:

1. *Potassium dichromate.*—Dissolve 2.1295 grams in one liter of distilled water. Use paraffined corks and keep in a dark place.

2. *Ferrous ammonium sulphate.*—Dissolve 5.6773 grams in one liter of distilled water. Before putting reagent in the bottle wash the bottle out with concentrated hydrochloric acid, leaving a trace lining the surface of the bottle. This prevents the growth of molds. Keep reagent in a dark place.

3. *Potassium permanganate.*—Weigh out slightly better than 0.4577 grams of crystals. Dissolve them in 200 cc. of water and boil down to approximately 50 cc. to remove organic residues. Dilute to 1000 cc. and titrate against 5.0 cc. of the standard ferrous ammonium sulphate until a faint pink color appears. Dilute the potassium permanganate solution until exactly 1.0 cc. of it is equivalent to 1.0 cc. of the ferrous ammonium sulphate.

4. *Sulphuric acid, 18 normal.* (Add equal parts of concentrated sulphuric acid and water to get 18 normal.)

The potassium dichromate should now for each cubic centimeter be equivalent to 3.0 cc. of the ferrous ammonium sulphate and consequently 3.0 cc. of the potassium permanganate. Also, each cubic centimeter of the potassium dichromate should be equivalent to 0.5 mgm. of alcohol.

ARSENIC

Since the advent of the Marsh test and consequently an easy method of arsenic detection, poisonings by the use of this metal have assumed a greatly reduced rôle and now arsenic usually is found in patients obtaining the metal through some industrial hazard. At this hospital there have been in a 23 year period only 27 cases of arsenic poisoning, representing 7.4 per cent of all cases of poisoning. Of these 60 per cent were from arsphenamine and its derivatives. There are at present several excellent methods for the estimation of arsenic, but for practical purposes and simplicity the Reinsch[6] test is excellent. If arsenic is found in a material upon examination by means of this method, it is at once apparent that the patient has been exposed to arsenic and consequently arsenic poisoning must be considered. The procedure is easily carried out and the results are very reliable. It is as follows:

1. Concentrate the urine, gastric juice or vomitus to one fourth the original volume and add one sixth volume of pure arsenic free hydrochloric acid. If feces or other solids (organs etc.) are used, dissolve in a measured volume of distilled water after breaking up into minute particles and add the hydrochloric acid as for urine.

2. Place in the solution a small strip (2 x 1.5 cm. in area) of pure arsenic free, polished copper foil and heat the solution to boiling for 2–3 minutes. Arsenic will deposit on the copper as a steel gray, purple or black deposit. If pentavalent arsenic is suspected, add a few crystals of ferrous sulphate before boiling.

3. Wash the copper strip with water, alcohol and ether and dry between filter paper. If arsenic is present, there will be a purplish black deposit on the copper. However, the deposit may be very faint, and a confirmatory test should always be done.

4. As a definite confirmatory measure cut the foil into small bits and place in a reduction tube (a small glass tube about 5 mm. × 10 cm.). Hold the tube at a 45° angle and apply heat at the sealed end which also contains the bits of

foil. As the foil becomes hot the arsenic sublimes and deposits on the cooler portions of the tube in the form of octahedral crystals easily seen with the low power of the microscope. These crystals should be in a well defined form, the larger ones being nearer the heat and the smaller onces further up the tube. This should eliminate errors due to possible antimony or mercury. All oxidizing agents must be absent as they destroy the test.

3. If these are present, evaporate the material to dryness with excess hydrochloric acid in order to expel nitric acid and any free chlorine, then take up in water, acidify with hydrochloric acid and carry on in the usual manner. This test will detect 0.0065 mgm. of arsenic. Many of the organic arsenicals however do not respond to this test, and for them a preliminary treatment of the sample must be carried out prior to the adding of the copper foil. The procedure of Vitali[7] as given by Webster[8] is excellent.

As an example of the value of this test in diagnosis, the following case is given.

Case 1. F. G. No. 51133, a 67 year old English sheet metal worker, entered the hospital because of indigestion and vomiting of nine weeks duration. The onset of his illness was insidious, beginning with slight nausea, which gradually grew more severe and he began to vomit. Following the vomiting he developed diarrhoea, anorexia, weakness and loss of weight of twenty-three pounds occurred. Nine days before admission he was seized with a severe chest pain and became progressively more dyspnoeic. Physical examination revealed arteriosclerosis of considerable degree. Heart and lungs negative, B. P. 110/70, blood hemoglobin 110 per cent, red blood cells 5,230,000. A diagnosis of coronary sclerosis and angina pectoris was made. The question of arsenic poisoning was raised, and a Reinsch test done upon a specimen of urine revealed the presence of arsenic in quantities well above normal. Undoubtedly some of his symptoms could be explained by a mild degree of arsenic intoxication. (An analysis of the arsenic content of such a patient's hair would give much more of the metal, as it is deposited to a certain extent in the hair. This source can also be used to show an arsenic exposure which has taken place three to four weeks previously.)

BARBITAL COMPOUNDS

Since the synthesis of barbital in 1904 and phenobarbital in 1913 there have been numerous poisonings and not a few deaths from their use. Owing to the ease with which the general public can procure the barbiturates there is not likely to be any great decrease in the number of patients admitted because of barbiturate poisoning, and indeed there appears to be a slight tendency for them to increase. As a cause of poisoning in the Peter Bent Brigham Hospital, barbiturates were second only to lead, with a

total of thirty-six cases, or 10 per cent of all cases of poisoning over a 24 year period.

Consequently barbiturates occupy an important position in the field of toxicology. Koppanyi et al.[9,10] have rendered a valuable service in making available a quite accurate and yet not too tedious a test for the barbiturates based upon the cobalt acetate reaction. The procedure which follows is simple and can be carried out in any hospital laboratory by a competent technician.

1. Concentrate the urine, gastric contents, cerebrospinal fluid etc. over a water bath to about one fourth of the original volume and acidify the solution with dilute hydrochloric acid (6N).

2. Pipette 2.0 cc. into 8 grams of plaster of Paris, thus making a completely dry powder, mix well and pulverize. Transfer the powder to an extraction thimble. A Whatman thimble, ether extracted,* gives good service and can be used several times.

3. Connect to a reflux condensor and add through the condensor 10 volumes, (usually 20 to 30 cc.) of chloroform, so that the chloroform runs over and through the plaster of Paris mixture in the thimble.

4. Reflux at gentle heat for 30 minutes, filter the chloroform extract and concentrate over a water bath to 2.0 or 3.0 cc.

5. Remove a 1.0 cc. portion of the extract and add 0.05 cc. of a 1 per cent cobalt acetate solution, mix and add 0.3 cc. of 5 per cent iso-propylamine. A reddish violet color develops. At this point the amount can be determined easily with the photocolorimeter or by comparing the sample in a microcolorimeter against prepared standards containing 2.0, 1.0, 0.5, 0.3, and 0.15 mgm. of barbital respectively dissolved in chloroform to which 0.05 cc. of cobalt acetate and 0.3 cc. of iso-propylamine have been added. There must be no water nor moisture on any of the apparatus as a trace of moisture quickly destroys the test. Be sure to multiply by the correct factor which depends on the various concentrations and dilutions used. *Never use more than 1.0 cc. of aqueous solution to 4.0 grams of plaster of Paris.*

Reagents:

1. *Cobalt acetate.*—1.0 gram dissolved in 100 cc. of absolute methyl alcohol.
2. *Iso-propylamine.*—5.0 cc. in 95 cc. of absolute methyl alcohol.†
3. *Plaster of Paris.*—4.0 grams to 1.0 cc. of aqueous solution.
4. *Ether extracted thimbles.*—Whatman Company.

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* Suggested to the author by Dr. John Plummer of the Evans Mem. Hospital.

† Eastman Company, Rochester, N. Y.

A short cut, which gives good qualitative results, can be done by evaporating the material to dryness over a water or sand bath then extracting the residue with 10 volumes of chloroform, after which the chloroform is filtered and concentrated to 1–2 cc. The cobalt acetate and iso-propylamine are added as above. The reddish violet color should appear if barbiturates are present. The following cases are examples where the test proved of value.

Case 2. S. G., a 40 year old housewife, was brought to the hospital in coma. Relatives stated that she had been taking phenobarbital because of insomnia and that for some time she had been depressed. On occasions she would become quite morose. On the evening of entry she was found in a stuporous condition lying in the bathtub. On the bathroom floor there was an empty phenobarbital box. In removing her from the bathtub she was accidentally struck quite a severe blow on the head. The stupor increased, she became comatose and was rushed to the hospital where examination revealed a middle aged female in a deep stupor. Temperature, pulse and respiration 100°, 128 and 50 respectively. There was a small red contused area over the right temporal region. Pupils small, equal, regular and reacted to light; lips cyanotic and the tongue dry; heart normal but a few râles could be heard at the lung bases; reflexes feeble; laboratory studies normal; blood pressure 90/60. A sample of urine submitted to analysis revealed 0.3 gram of phenobarbital. Subsequent specimen over a four day period yielded a total of one gram. In view of the nature of phenobarbital excretion this amount represented only about 1/10 of the amount ingested or 10 grams, which is quite close to the fatal dose. Patient gradually regained consciousness and in view of her history and suicide attempt she was transfered to a psychopathic hospital.

Case 3. G. W., a 24 year old chauffeur, was brought to the hospital in coma. Friends said that he had been under severe mental strain and that for the past two days had been consuming considerable alcohol. On the evening of entry he was found in an unconscious state, lying on the bathroom floor. Beside him was an empty phenobarbital bottle and a suicide note. He was rushed to the hospital, where examination revealed a well developed and nourished male in a somnolent state with a definite odor of alcohol to his breath. Upon shaking or speaking loudly he could be raised from the deep sleep and would say a few unintelligible words, but immediately lapsed into the semi-stupor. Temperature 98°, pulse 82, respirations 23; pupils round, equal, normal in size and reacted sluggishly to light; heart and lungs negative; blood pressure 100/70. There were a few minor abrasions over the knuckles of the right hand and over the right knee. Reflexes normal. Routine laboratory studies normal. A sample of blood revealed 30 mgm. of alcohol per 100 cc., which indicated that he had been drinking quite heavily some time before entrance and that a good deal of his stupor might be on the basis of alcoholic narcosis. A catheterized specimen of urine (the first obtained from the patient on arrival) was subjected to analysis for barbiturates, but none were found. This examination was re-

peated several times and on various specimens of urine, but on no test was there more than a faint suggestion of any barbiturates. The empty bottle was obtained and washings from it were found to contain abundant phenobarbital. A question as to the validity of the patient's story naturally arose. He was given a 0.2 gram dose of phenobarbital and urine was collected after 6 hours which gave a very definite reaction for barbiturates. In view of the evidence thus obtained it became quite questionable whether he had ever taken the drug as stated. Subsequent history supported the idea that the note was faked in an endeavor to gain sympathy. Thus valuable information was gained of the patient's mental state, and he was treated accordingly.

BROMIDES

Since Magendie's[11] introduction of bromide into medicine in 1829 there has been an ever increasing use of it in many conditions. Owing to extensive advertising, the ease with which they can be procured and relative non-toxicity bromides have become a common household remedy and are used for every condition from "morning after" headaches to epilepsy. As a result, it is not surprising that cases of poisoning appear from time to time in a general hospital. Also, many patients present skin rashes as a result of bromide sedation, but give an entirely negative history as they are unaware that the medicine they are taking contains any bromide. A quite simple laboratory test readily indicates the basis of the difficulty. As for the other halogens, there are several methods for detecting bromides, and like its fellow members of the halogen series, bromine requires a rather elaborate procedure, if great accuracy is required. For ordinary purposes there are two or more quite simple methods of detection. The procedure used by Hauptmann[12] and modified by Katzenelbogen et al.[13] for their studies on the hematoencephalitic barrier is easily adapted to a hospital laboratory and has given excellent results in our hands.

1. Pipette 2.0 cc. of serum into a test tube and add 4.0 cc. of 0.85 per cent sodium chloride.
2. Add 1.2 cc. of 20 per cent trichloracetic acid, allow to stand for 30 minutes, then filter.
3. Pipette 2.0 cc. of the filtrate into a test tube and add 0.4 cc. of 0.5 per cent acid brown gold chloride solution.
4. Mix well and read in colorimeter or compare with standards.

Calculations:

$$\frac{10 \times \text{factor}}{R} = \text{mgm. of sodium bromide per 100 cc. of blood}$$

Set standard at 10. R = reading of unknown. Any value above 30 mgm. is evidence of bromide medication. A stock solution of sodium bromide is prepared containing 166.8 mgm. diluted with physiological salt solution to 100 cc. from which by dilution the following factors are obtained.

Dilute	1.0	cc.	of	stock	solution	to	10.0	cc.	with	NaCl =	50 factor
"	2.0	"	"	"	"	"	"	"	"	=	100 "
"	3.0	"	"	"	"	"	"	"	"	=	150 "
"	4.0	"	"	"	"	"	"	"	"	=	200 "
"	5.0	"	"	"	"	"	"	"	"	=	250 "
"	6.0	"	"	"	"	"	"	"	"	=	300 "
"	7.0	"	"	"	"	"	"	"	"	=	350 "

When smaller amounts of bromide are suspected the smaller factors are used. To 5 cc. of the above chosen dilution (depending on the concentration of bromide suspected) add 1.0 cc. of trichloracetic acid, 1.2 cc. of 0.5 per cent gold chloride, mix and compare with the unknown sample.

Spinal fluid. Add 0.8 cc. of 20 per cent trichloracetic acid to 4.0 cc. of spinal fluid. Let stand for 30 minutes, then filter. Pipette 2.0 cc. of the filtrate into a test tube and add 0.4 cc. of 0.5 per cent gold chloride. Mix and compare against chosen standard.

Calculation:

$$\frac{10 \times \text{factor}}{R} \times .66 = \text{mgm. of sodium bromide per 100 cc.}$$

The following case illustrated the value of the test in confirming a diagnosis.

Case 4. T. G., a 33 year old reporter, entered the hospital because of generalized tremor and hallucinations of six days duration. For the past six months he had consumed considerable alcohol. Eight days before admission he began to complain of severe headaches and talked incoherently. He became mentally cloudy and developed a generalized tremor. Bromides in moderate dosage were prescribed by his physician, but because of the poor response to the medication he increased the dosage so that it was later estimated that he had taken between 90–100 grams of triple bromides in a period of five days. Two days before admission he refused all food and sank into a deep stupor. Examination upon admission revealed a man in deep stupor whose temperature was 101°, pulse 100 and respiration 24. The skin over the body and especially over the face exhibited a pustular rash. Pupils equal, regular and reacted sluggishly to light, heart negative, blood pressure 130/80. Lungs, diminished resonance at the right apex, respiration shallow, accurate evaluation of lung condition impossible. Reflexes absent. A diagnosis of delirium tremens and questionable bromide intoxication was made. Patient lived only a few hours

and died on the day of admission. Autopsy refused. However, a sample of blood was obtained and analysis for bromides gave 413 mgm. per 100 cc., a definite proof of bromide intoxication and certainly an important factor in causing the patient's death.

IODIDES

The importance of this halogen in medicine has steadily increased since its introduction by Magendie[14] in 1821. It has been advanced further in use by the work of Marine and his associates[15, 16] in advocating it for school children and residents in the so-called goitrous areas, and by Plummer's[17] discovery of its value in bringing about a remission in hyperthyroidism. As a result of this widespread use of the drug there are constantly appearing patients who have skin rashes or mucous membrane irritation as a result of the drug. Often it is quite difficult to be certain whether a rash is a result of iodide intake or not and this is especially so if the patient gives a negative history or does not know the nature of the medicine he is taking. It is occasionally desirable to know whether the tetraiodophenolphthalein of a gall bladder series has been absorbed or not. Rudisill and Hemingway[18] have shown that if 5 mgm. of iodine is found in the urine there is definite proof the dye was absorbed and that the difficulty lies in the gall bladder. A simple analysis may in this way save the time and expense of a repeat film. The method employed is that described by the above writers and is as follows:

1. To 50 cc. of urine add 1 gram of sodium hydroxide and heat over a sand bath until about 10 cc. remains.
2. Add to the residue one gram of powdered potassium nitrate by sprinkling over the surface.
3. Continue evaporation to dryness and fuse at moderate heat until the carbon has disappeared, and the fused material is white.
4. Cool and dissolve in 20 cc. of water.
5. Filter and acidulate the filtrate with dilute 1:6 sulphuric acid until acid to litmus.
6. Add 0.5 grams of sodium nitrite and place the whole solution in a separatory funnel.
7. Extract the mixture with 5.0 cc. of chloroform. A pink color in the chloroform indicates iodine. A deep purplish pink represents about 5.0 mgm. of iodine, a moderate pink 2.5 mgm. and a faint pink about 1.25 mgm.

A short cut may be employed when non-organic iodine medication is suspected by acidulating the urine with dilute 1:6 sulphuric acid and adding 0.5 grams of sodium nitrite, followed by extraction with chloroform in a separatory funnel. If iodine is present, the chloroform layer will have a pink discoloration.

LEAD

Very little need be said about the extremely important rôle this heavy metal plays in the field of toxicology. Of 363 patients admitted to the Peter Bent Brigham Hospital in the years 1913–1936 for poisonings of various natures there were 64 with lead poisoning, or 17.6 per cent, thus leading the next highest, barbiturates, by 8 per cent. The desire for accurate analytical data arises occasionally and many cases of poisonings are missed if such procedures are not carried out. Unfortunately there is at the present time no simple and yet accurate method for the estimation of lead. The procedure outlined by Fairhall[19] gives quite excellent results in urine and organic material. Unless there are numerous occasions for the use of this procedure, it is better to send the specimen to some central laboratory where large numbers of determinations are done because the standard solutions require considerable care in making and are liable to deteriorate after a time and require rechecking.

MERCURY

This metal formerly occupied an important position among the list of poisons, but at the present time it is only rarely a cause for hospital admission. Of the patients admitted for poisonings between 1913–1936 at the Peter Bent Brigham Hospital there were 30 patients with mercury poisoning, or 8.3 per cent. In the years between 1915–1925 there were 23 patients, or 76.7 per cent of the group, while for the period 1925–1936 there were only 6 cases, or 24.3 per cent. This is a remarkable drop. An important factor in bringing about such a state is the legal restraint on the promiscous selling of the dangerous bichloride, the care taken to label it properly in an easily noticable manner and the use of odd shaped and colored tablets. An examination for

mercury is occasionally of value as a check in patients where there is a question of mercury poisoning.

The Reinsch[20] test adapted for mercury examination is quite simple and very accurate. The procedure is quite analogous to that of arsenic estimation, and the same steps are carried out to where the copper foil is placed in the sample to be analyzed. The mercury deposits on the copper foil as a gray or silvery deposit.

1. Remove the foil, wash with water, alcohol, ether and dry between filter papers.
2. Cut the dried foil into small pieces and place in a reduction tube as described under arsenic.
3. Heat the lower end of the tube over an open flame until the copper glows slightly. The mercury sublimes and collects on the cooler portions of the tube in the form of typical globules of mercury (low power microscope).
4. Now shake out copper foil and place in the tube a crystal of iodine and stopper with cotton. Allow to stand a short time and the sublimate will become red owing to the formation of mercuric iodide. Nitric acid and oxidizing agents destroy the test. It is very sensitive and will detect the presence of as little as one one hundred thousandth of a grain.

MORPHINE

Of all the alkaloids the ones most often requiring detection by the physician in a general hospital are morphine and codeine. The rigid enforcement of the Harrison narcotic laws has certainly lead to a marked diminution in the numbers of addicts and has curtailed very effectively the promiscuous use of these drugs. Since the opening of the Peter Bent Brigham Hospital in 1913 there have been only 5 patients with acute codeine or morphine poisoning and 32 patients who were classified as addicts. As a consequence of such strict regulations those patients who are addicts are exceedingly secretive and remarkably clever in keeping the physician ignorant of their habit. Sometimes the information can be quite readily obtained by a simple urine test which indicates morphine intake and consequently raises the question of addiction. Codeine does not present such a problem, but at times it is of value to know whether a patient has been or is taking the drug.

The following simple test of Marquis (21) is easily carried out.

1. Evaporate urine or stomach contents to dryness in a white porcelain evaporating dish.

2. With a glass rod transfer a drop of the sulphuric acid-formalin reagent to the dried residue. If morphine is present an intense purple-red color changing to violet and then pure blue develops. Codeine does not give the original purple but strikes a violet and then changes to blue. A marked reaction is given by as small amount as .02 mgm. of morphine. The sulphuric acid-formalin reagent is prepared fresh each time by adding two or three drops of 40 per cent formaldehyde (formalin) to 3 cc. of pure concentrated sulphuric acid.

A very good confirmatory biological test is the mouse injection of Herrmann and Heinekamp[22, 23] where a neutral or slightly alkaline sample of the material suspected is injected under the skin on the back of a white mouse of 20 grams weight. The symptoms are characteristic and consist of lordosis of the back, slight spastic paralysis of the hind legs and the tail assumes a quite characteristic position raising in a S shaped curvature and finally lying over the back with the tip being over the region of the ears. The animal gives a marked response to even the slightest stimuli and appears quite restless. A dose of a few milligrams may bring about a reaction for several hours.

Case 5. H. C., a 39 year old Chinese laundryman, entered the hospital in a marked state of emaciation. He had been on a grossly inadequate diet for six months. Five weeks before entry the skin over the backs of his hands and over his shins had begun to be red, scaly and rough. Examination upon admission revealed severe emaciation; areas of skin quite suggestive of pellagra were present over the shins and backs of the hands. Heart and lungs negative, B.P. 110/60. Prostatic outline was obliterated by a mass which caused the patient severe pain when palpated. Laboratory studies showed a blood hemoglobin of 40 per cent, red blood cells 1,470,000 and white blood cells 1,350. Some suspicious brown pellets were taken from the patient who upon further questioning admitted that he chewed opium. In the meantime a sample of his urine had been analyzed which gave a strong reaction for morphine. A confirmatory mouse test was characteristic. After a prolonged hospital sojourn he was discharged to a State Hospital in a very much improved condition.

SUMMARY

An attempt has been made to review in a brief general way the more common toxicological procedures and to indicate in what ways they may prove of use in a general hospital. Five case reports illustrate the value of the various methods. Details of analytical procedures for alcohol, arsenic, barbiturates, bromides, iodides, mercury and morphine are given.

I wish to acknowledge the encouragement and many valuable suggestions made by Dr. H. A. Christian. To Dr. John Plummer of the Evan Memorial

Hospital I am indebted for sound advice and excellent suggestions in regard to the method of barbital analysis.

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PREPARATION OF THE AUTOPSIED BODY FOR EMBALMING

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Received for publication February 16, 1938

Two major objections to post-mortem examination are advanced by the embalmer. The first of these arises from the fact that, to restore an autopsied body, additional time, labor and technical skill are required, for which no definite compensation is provided. The second and the more serious objection lies in the well founded fear that autopsy may interfere with proper embalming of the body and indeed, at times, make it well nigh impossible for the embalmer to restore the likeness of the subject. Many a contemporary embalmer is said to be unprepared in the technique of properly restoring autopsied bodies. To him, an improperly performed autopsy is a curse. It is, therefore, but natural for him to attempt to adversely influence the decision of the bereaved relatives. To the physician, this seems an unwarranted interference but to the embalmer a legitimate self defense. The situation naturally creates an atmosphere of mutual distrust and antagonism.

To adequately meet these just objections it becomes incumbent upon the pathologist to make every reasonable attempt to conduct these examinations in a manner least offensive to the embalmer and the funeral profession.

Briefly outlined, an acceptable post-mortem examination from the embalmer's standpoint should fulfill the three requirements, namely, (1) the properly executed incisions; (2) the proper care of the body and its principal arterial trunks and (3) the proper disposition of the discarded visceral organs, tissue fragments, etc.

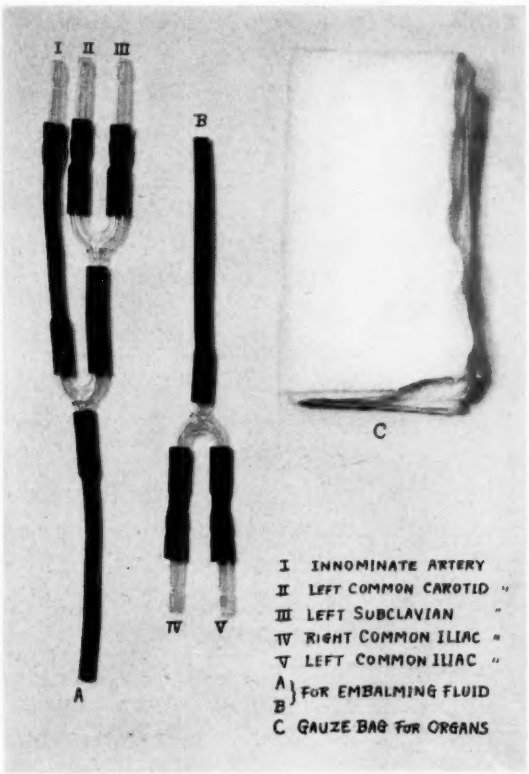

FIG. 1. The glass connecting tubes with rubber tubings, attached, ready to be inserted and tied to the main arterial trunks as illustrated in figure 2. In tying the glass tubes to the vessels, an inexpensive holder, (automobile mechanical plier, cost 8¢) may be advantageously used to hold them in place. Note also a sack or bag made of gauze to put visceral organs and detached tissues.

THE INCISIONS

(a) The incision over the trunk should be made in a Y shape. In adult females, the short limbs of the Y should be directed beneath the breasts.

(b) The scalp incision should be so made from behind the ears as to be

easily hidden by the hair and the pillow and the cut through the frontal bone be kept well behind the hair line.

(c) To maintain the head and neck in their proper position, it is advisable not to disarticulate the sterno-clavicular joints but to preserve the continuity of the clavicles to provide the necessary support to the neck. For this purpose,

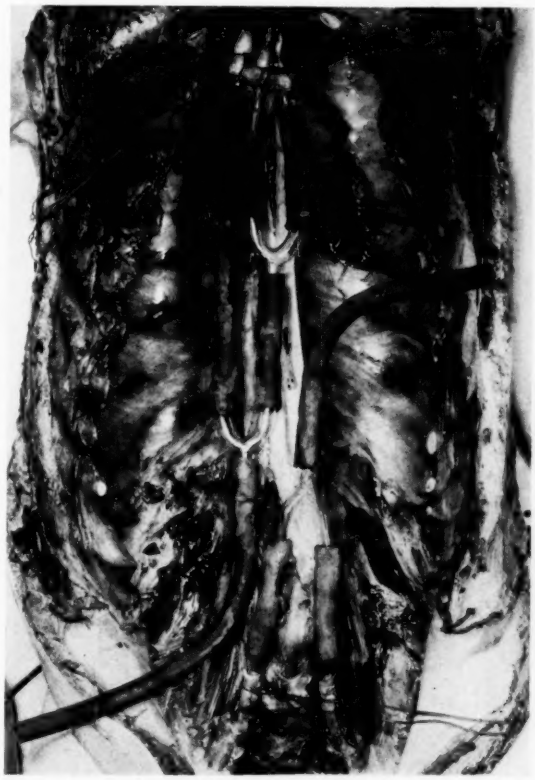

FIG. 2. An actual view of a body cavity after routine autopsy, showing the connecting tubes tied to the principal arterial trunks.

the anterior thoracic plate should be cut transversely through the manubrium, one or two centimeters below the sternal notch.

THE CARE OF THE BODY AND THE ARTERIAL SYSTEM

The proper preservation of the body is imperative for effective embalming.

(a) The experimental evidence[2] indicates, the body is best stored in a temperature of from thirty-five to thirty-seven degrees, Fahrenheit.

(b) Freezing refrigeration of the body for a prolonged period, especially

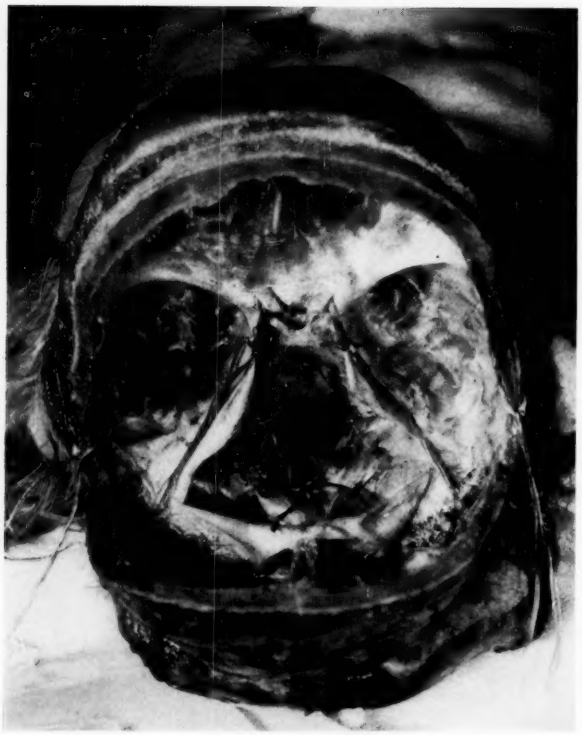

FIG. 3. A view of the skull opened, showing the internal carotid and vertebral arteries tied and the perforation made along each angle of the opened skull plate to allow a piece of cord to hold the skull cap in place.

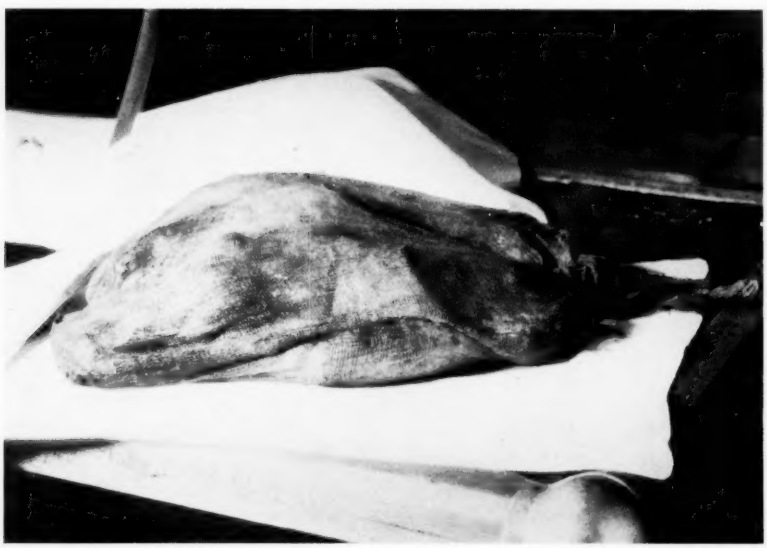

FIG. 4. A gauze sac filled with tissue remnants and discarded organs, tied and ready to be deposited into the dried body cavity.

after autopsy, has been found to cause technical difficulties usually encountered in a frozen body.

(c) Leaving the body in room temperature must, of course, be religiously avoided.

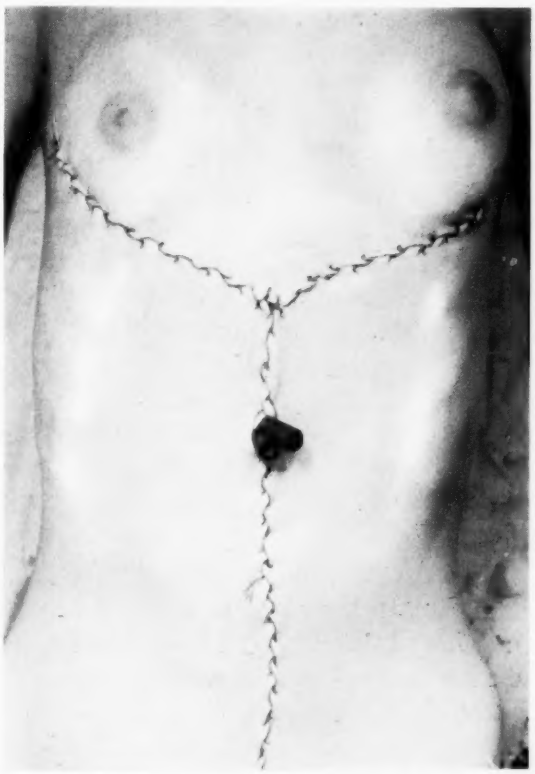

FIG. 5. A routine incision on the body of a young female, tightly sutured. Note the course taken by the incision. Two open ends of the rubber tubings, one connected to the vessels of the arch and the other to the common iliac arteries, are anchored to the incision. Embalming fluid may be injected through these openings.

(d) In this connection, it should be stated that once the permission is granted, unnecessary delay in the performance of the examination or in the delivery of the body to the embalmer should be avoided. Such delay may add further difficulties to proper embalming.

The proper care of the severed arterial trunks is perhaps the most important, one which "makes or breaks" the embalmer.

(a) The arch of the aorta and the arterial trunks arising there-from should be preserved in toto, or if cut, each cut end be anchored by a piece of cord to prevent its retracting out of reach so that the canulas may be readily inserted.

(b) Similarly, the common iliac arteries should be held, and the internal iliacs ligated to prevent the escapes of the fluid.

(c) If the skull is opened, the internal carotid and vertebral arteries at the base of the skull are to be ligated.

(d) Special precaution should be taken to safeguard the carotid arteries in case the exploration of the neck is undertaken.

In 1936 Peasely[1] published "A Method of Preparing the Body After Autopsy" in which he described a technique which makes possible easy and adequate embalming of autopsied bodies. For three years, he had made a routine use of this method on more than one hundred bodies. As a result he states that, "the opposition from the undertaken has been replaced by an agreeable coöperation."

Although the procedure is simple and inexpensive (about fifty cents per body for the material) and overcomes much of the embalmer's objections to autopsy, neither the pathologist nor the medical profession has apparently shown sufficient interest in its routine and universal application. While the expenditure of fifty cents for each autopsy may amount to a prohibitive sum in the autopsy service of large public hospitals, the average private hospital may well afford to adopt this method as a routine measure in its autopsy room.

I have adopted the method with slight modification and applied it on approximately seventy-five bodies. The enthusiastic response of the local funeral directors and embalmers has been attested by their unqualified assurance of the closer coöperation and assistance in obtaining the permission for post-mortem examination. This has also removed their principal argument against autopsy, one which they have effectively used to influence the decision of the relatives, namely, the contention that the proper embalming and restoration of the body is difficult and sometimes impossible after the "mutilating" examination.

The technique of Peasely's somewhat modified by me is given in the accompanying illustrations with explanatory legend.

THE GENERAL CARE OF THE BODY

(a) A well conducted post-mortem examination must include the meticulous care of the autopsied body and proper disposition of the remaining visceral organs and tissue fragments. The bits and tags of tissues are carefully cut away from their attachments. All free tissue fragments are gathered. These, together with all discarded portions of the visceral organs, are placed in a gauze sack. Excess fluid is then drained out and the sack placed in the body cavity which has been thoroughly sponged of fluid and blood. This enables the embalmer to handle the discarded contents of the body with ease and dispatch, eliminating another of the disagreeable part of the work.

(b) A partial post-mortem examination through a limited space such as a surgical incision is not only seldom satisfactory to the pathologist but hardly welcome by the embalmer, since it creates, in the disturbed area, arterial leakage which interferes with proper embalming. The embalmer prefers a complete autopsy to a limited and often mutilating examination which is frequently carried out by the clinician himself.

(c) A light arterial embalming alone seldom interferes with the satisfactory examination of the body, except where the complete bacteriological data are desired or a medico-legal question is involved. I am inclined to let the embalmer proceed with his injection whenever practicable to do so.

(d) Needless to state that any other small considerations which make the embalmer's task easier and more pleasant, such, for example, as tightly suturing all incisions to prevent leakage of fluid or tying in place the skull cap through the small perforations made along the free borders of the opened skull and the like, would naturally tend to further promote the good will of the funeral profession.

During the autopsy, it is customary to cover the face of the dead with a moistened towel. I have found it appropriate to spread a wet sheet over the entire lower extremities below the symphysis pubis. Frequent and liberal use of running water and sponge is necessary to maintain the clean technique.

Post-mortem examinations can be conducted with proper regard for the dead and with as clean a technique as conditions permit. This requires no further emphasis.

CONCLUSIONS

One of the best means of obtaining more autopsies is to first obtain the good will of the funeral profession. The best way to secure that good will is for the pathologist to offer his sympathetic coöperation to the embalmer (1) by showing proper appreciation of the difficulties the improperly autopsied body may bring to him and (2) by adopting such means and methods in the performance of autopsy as to eliminate or minimize these difficulties.

A technique of properly preparing the autopsied bodies is described making the special emphasis on the care of the arterial system which is of the first and foremost importance in the art of embalming.

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CULTURE METHOD FOR ISOLATION OF TUBERCLE BACILLI FROM CONTAMINATED MATERIALS

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Received for publication February 10th, 1938

I. PREPARATION OF POTATO-EGG MEDIUM

The medium to be described has been in use for the past two years in the laboratories of three New York State Tuberculosis Hospitals* with satisfactory results and is easily prepared. Two

* The Herman M. Biggs Memorial, the Mount Morris, and the Homer Folks Tuberculosis Hospitals.

important factors in the preparation of any egg medium are a minimum amount of heating and the prevention of the loss of water from the medium.

1. All glassware and utensils coming in contact with the medium are sterilized in the autoclave for thirty minutes at fifteen pounds.

2. *Glassware and utensils.* Test tubes, six by three quarter inches, stoppered with non-absorbent cotton, and with number eight long cork stoppers; a 1000 cc. Squibb separatory funnel to which is attached a short length of rubber tubing with a glass tip and spring clamp; an egg beater; a 500 cc. and a 25 cc. graduate; a three quart pot with cover; a four inch glass funnel and a dozen foot square pieces of gauze.

3. *Potato-extract.* To 1000 cc. of 12 volume per cent glycerine water is added 250 grams of thinly sliced, freshly peeled potato with a minimum exposure to air. This is autoclaved with the glassware.

4. *Eggs.* Fresh eggs are washed in water and immersed in 70 per cent alcohol for two hours.

5. *Malachite-green oxalate.* Sterile 2 per cent aqueous solution.

6. *Potato-egg.* Into a three quart pot are poured 500 cc. of whole egg, 100 cc. of egg yolk and the mixture is beaten well. Then 400 cc. of potato-extract (3) cooled to about 50°C. and 12.5 cc. of a 2 per cent malachite green (5) are added. The mixture is well beaten with an egg beater and then filtered through several thicknesses of sterile gauze into the tubing funnel. Ten to 12 cc. are filled into each tube, the tube is stoppered with a cotton plug, slanted in the inspissator or Arnold sterilizer and heated at 85°C. for exactly one-half hour. If the heating is done in an inspissator the tubes are stoppered with cork stoppers to prevent the loss of water from the medium during inspissation. If the heating is done in an Arnold sterilizer the tubes are stoppered soon after they are removed from the sterilizer. One heating is sufficient if sterile precautions were maintained during the preparation of the medium. The tubes are then incubated for three days to test for sterility.

II. PREPARATION OF SPECIMENS

The importance of the proper preparation of the specimen for culturing cannot be overemphasized. Badly contaminated materials need somewhat longer treatment with alkali or acid than those which are only slightly contaminated. Occasionally saprophytes may overgrow the entire surface of the medium in spite of treatment. On the other hand one may occasionally lose a guinea pig from secondary infection. The following methods have been used for preparing specimens for culture.

1. *Sputum.* Petroff's Sodium Hydroxide Method. Sputa are mixed with an equal amount of a 3 per cent sodium hydroxide, placed in a shaking machine for thirty to forty-five minutes. Thick purulent sputa are first diluted with an equal volume of sterile physiological salt solution before adding the sodium hydroxide. If a shaking machine is not available the specimens are placed in the incubator at 37°C. and shaken by hand every ten minutes. They are then neutralized to phenolphthalein with 15 per cent (by volume) sulfuric acid. When the more mucoid type of sputa are being neutralized one drop of acid is added after the faint pink end point is reached so that the solution is just colorless. Heavy purulent sputa are only partially neutralized, sulfuric acid is added until the specimens are no longer gelatinous but appear watery when shaken. They are then centrifuged and one or two drops of a 3 per cent hydrochloric acid is added to the sediment. The amount of acid depends on the volume of sediment.

Corper's Sulfuric Acid Method: To sputum is added an equal volume of 6 per cent sulfuric acid (3.2 cc. of concentrated sulfuric acid to 97 cc. of water) mixed well and placed in a shaking machine or incubator for twenty to thirty minutes. Then nine volumes of sterile physiological salt solution are added and finally centrifuged in a sterile tube covered with a rubber cap.

2. *Urine.* Twelve to 24 hour specimens are collected in sterile containers and kept cold during the period of collection to prevent overgrowth of bacteria. When collecting specimens from female patients results are generally much more satisfactory when catheterized specimens are obtained. The specimens are then

centrifuged or if the amount is too large they are first treated with tannic acid according to Petroff's method.

Ten cubic centimeters of sterile 1 per cent tannic acid is added to every 1000 cc. of urine and after mixing is placed in the refrigerator overnight to allow the precipitate to settle. The supernatant fluid is then siphoned off without disturbing the sediment and the remaining portion is centrifuged in sterile tubes. The sediment obtained is treated with sulfuric acid or sodium hydroxide as described for sputum above.

3. *Pleural fluids.* Pleural fluids are collected in sterile bottles containing sodium citrate to prevent coagulation of the fluid. The fluid is then centrifuged at high speed for one hour and the sediment, if it does not contain other microörganisms, is seeded directly or after treatment for ten minutes with 6 per cent sulfuric acid. Some untreated sediments digest the medium which may inhibit the growth of bacilli. Fluids containing other microorganisms are treated with sodium hydroxide or sulfuric acid the same as sputum. Fluids from cases of pneumothorax do not often coagulate so that they need not be collected with sodium citrate. Otherwise the treatment is the same.

4. *Spinal fluid.* To spinal fluids sterile 1 per cent tannic acid is added drop by drop until a faint opalescence appears. The fluid is then centrifuged for one hour and after making a smear the sediment is seeded directly or after treatment with 6 per cent sulfuric acid for ten minutes as for pleural fluids.

5. *Pus.* Swabs, gauze dressings or cotton plugs containing pus are immersed in sterile physiological salt solution to dissolve out the pus and then treated with sodium hydroxide or sulfuric acid the same as sputum.

6. *Feces.* Feces are prepared according to Petroff's sodium-chloride method or treated directly with sodium hydroxide or sulfuric acid. Much longer treatment with acid or alkali is required to destroy the contaminating microörganisms. Two tubes are seeded from the sediments obtained after 30, 45, 60, and 120 minutes treatment. Frequently one tube from the 30 or 45 minute period remains uncontaminated.

7. *Blood.* To 40 cc. of sterile distilled water is added 1 cc. of

3 per cent sodium hydroxide and 10 cc. of the blood. The solution is poured into a sterile centrifuge tube and centrifuged for one hour. The sediment is then treated with an equal volume of 6 per cent sulfuric acid and after standing for ten to 15 minutes is diluted with nine volumes of sterile physiological salt solution and centrifuged for 30 minutes. If the sediment obtained after the first centrifuging is too bulky more alkali is added after mixing, the solution is again centrifuged. There should be a very small amount of sediment if the right amount of alkali is present. Some bloods require slightly more alkali than others.

Comment. A certain amount of experience is necessary in choosing the chemical and the length of time for the preparation of specimens for culturing. Treatment with alkali will generally result in a smaller amount of sediment than acid treatment, thus increasing the chances for demonstrating small numbers of tubercle bacilli. It must be remembered that the shorter the treatment with either reagent consistent with the destruction of secondary microörganisms the more likelihood there is for obtaining a growth of the tubercle bacillus.

RAPID HEMATOXYLIN-EOSIN STAINING OF FROZEN SECTIONS*

A. M. YOUNG

From the Laboratory Department, Mt. Sinai Hospital, Cleveland, Ohio

The following satisfactory technic for rapid staining of frozen sections with Hematoxylin and Eosin has been in routine use at Mt. Sinai Hospital of Cleveland since 1923.

FIXATION

Place the block of tissue in boiling 10 per cent formalin in a covered dish and boil for one to three minutes depending upon the thickness of the specimen.

CUTTING AND MOUNTING

1. Immerse in cold tap water, trim if necessary and cut on the freezing microtome at 15 to 20 micra.

* Received for publication, December 30th, 1937.

2. Transfer the sections to water in a large glass dish over a dark background. A crystallizing dish 18 cm. in diameter is very satisfactory.

3. Mount the selected section under water on a clean glass slide previously covered with a thin film of egg albumin.

DEHYDRATION

1. The section after being properly mounted on the slide is blotted with filter paper to remove water and to help fasten it to the slide.

2. The slide is then flooded with absolute alcohol (some is rubbed on the under surface with a finger) and blotted. This process is repeated three times to insure complete dehydration.

3. The slide is then flooded with celloidin which has been thinned to the proper consistence with a mixture of equal parts of absolute alcohol and ether. After about one half minute the slide is immersed in water.

STAINING

1. Place the slide on an electric hot plate and alum hematoxylin solution as employed in staining paraffin sections is superimposed over the section and allowed to steam for one minute. This step may be carried out on a staining rack by heating the under surface of the slide with the flame of a bunsen burner.

2. Intensify the stain by immersing the slide in 1 per cent ammonia water. Wash in tap water and counterstain a few seconds in 1 per cent eosin. Dehydrate rapidly in graded alcohols completing the dehydration by flooding with absolute alcohol and blotting three times.

3. The section is then cleared in about 10 seconds in xylol and mounted in balsam.

THE RHAMY FROZEN SECTION TECHNIC INTRODUCING A MODIFIED STAIN AND A METHOD FOR PERMANENT MOUNTS

J. M. FEDER

From The Department of Pathology, Anderson County Hospital, Anderson, S. C.

Received for publication January 20, 1938

About a decade ago, Rhamy[1] gave us a frozen section technic that was at once highly efficient and simple in application. We considered it a great improvement over any method then used and at once adapted it to our needs.

Unfortunately, the stain suggested by Rhamy was not as easily prepared as its simple formula would lead one to believe. Many

tried to reproduce his results and after a few trials and failures assigned the method to the discard. We found that we were unable to produce a satisfactory preparation every time. Realizing that we were working with saturated solutions and that the saturation point was governed by many varying conditions, a method of preparing the stain was sought in which powdered stain was used.

In our search, we laid down five cardinal principles that our finished product must meet: 1. Reproduction of the Hematoxylin-Eosin picture as nearly as possible with due consideration to be given to the type of dye used. 2. Rapidity of action. 3. Simplicity of preparation. We wanted a stain that could be prepared from the dye found in any laboratory. (Only the highest grade material could be used in making the Rhamy stain.) 4. Nuclear differentiation under the higher magnifications. 5. A stain that would be adaptable to permanent mounting from the original preparation.

After considerable experimentation we believed that the formula noted below most nearly approached the ideals set forth in our five point program:

Toluidine Blue	0.8 grams
Basic Fuchsin	0.3 grams
Eosin Y	0.4 grams
Alcohol 30 per cent	110.0 cc.

The preparation of the stain is carried out by measuring 33 cc. of 95 per cent alcohol into one graduate and 77 cc. of distilled water into another. The powdered stain is weighed and transferred to a glass mortar and the alcohol added until the stain is dissolved. The water is then added and the stain filtered. It is ready for immediate use and ripening is not required.

In the event that the red contrast stain appears too deep for the use of the individual worker, it can be corrected by reducing the amount of eosin and fuchsin added.

Staining Technic: 1. Fix fresh material in the usual manner. (We do this by the use of a vacuum apparatus. In this manner the hot fixing fluid is rapidly introduced throughout the tissue. Blocks of fresh tissue are placed in a 50 cc. flask and hot 10 per cent formalin added. This flask is connected to vacuum pump and kept under negative pressure for one minute.)

2. The tissue is rapidly washed and sections cut on freezing microtome with

customary technic. We do not seek extra thin sections. Experience has taught us that a 15 micron section showing the structure in its continuity is to be preferred to a 5 micron section that is crumbled and distorted.

3. Sections are floated from the knife into 4 per cent formalin.

4. The section is now floated onto a slide. We find it vastly superior technic to stain the section flattened on a slide to the use of a staining rod. The staining is much more uniform.

5. Drop onto the section a few drops of the modified Rhamy stain and allow it to remain for about 15 seconds or to a rapid count of forty.

6. Wash in water.

7. Remount section and blot away excess water.

8. Mount with a drop of mounting fluid consisting of equal parts of alcohol, glycerine and distilled water.

By using the above technic, we have been able to render a satisfactory report within four minutes from the time the material was received for examination.

Technic for permanent mounts: Satisfactory permanent mounts can be made in connection with the above method by following out the simple technical steps enumerated below:

1. After staining section and washing away excess stain, it is floated into a dish containing 95 per cent alcohol and allowed to remain there for about five seconds.

2. Remount on slide, wipe away excess alcohol and apply several drops of Diaphane Solvent.* Allow this to remain for one minute.

3. Wipe away excess Diaphane Solvent and mount in Diaphane, (green).*

Sections so mounted are permanent and only slightly inferior to those prepared in the orthodox manner. This procedure is greatly facilitated by the use of the Diaphane products.

By following this technic, we find it possible to clear the counter of the majority of work and render reports on the day it was received. It is possible to comfortably dispose of fifteen sets of tissue in an hour by this means and that about covers the work range of a moderate size institution. Of course we still have the tissues that will not be suitable for freezing and those requiring special study and they will be taken care of by the usual orthodox methods.

In using the Diaphane technic one does not have the shrunken, crumpled sections so often seen when an attempt is made to

* Diaphane products are secured from Will Corporation, Rochester, New York.

mount frozen sections permanently using the usual dehydrating methods.

CONCLUSIONS

An easily prepared frozen section stain has been presented together with technic of application and a rapid method of making permanent mounts.*

REFERENCE

(1) Rhamy Triple Stain for Frozen Sections. J. Lab. and Clin. Med. **15**: 490, 1930.

A METHOD FOR THE PREPARATION OF CURETTINGS FOR MICROSCOPIC SECTIONS†

I. MILTON WISE

The preparation of curettings for microscopic examination becomes a tedious task because of the necessity of handling each bit of tissue repeatedly while passing through the various dehydrating solutions to the stage of paraffin blocking.

I have tried the methods in use of putting the tissue on paper or in gauze sacs and running them through as one piece, but these methods have their disadvantages because bits frequently become dislodged and then each piece must be handled separately when the block is prepared.

For this reason I have adopted the following method that allows one block of tissue to be run through all stages.

* Since this article was written the method of making permanent mounts has been slightly modified as follows: Instead of 95 per cent alcohol for preliminary dehydration a mixture of equal parts of alcohol, glycerin and water is used on which the section is floated for a few minutes before mounting on a slide. A mixture of equal parts of Diaphane solvent and Diaphane is then dropped on, and allowed to remain 5 minutes. After wiping off the excess, apply a drop of Diaphane and a cover glass. The permanent mount technic has expediency rather than beauty to recommend it.

† Received for publication December 10th, 1937.

1. The curettings are received on moistened gauze, the tissue fragments picked out and put in a large test-tube with about 15 cc. of water, (Fig. I (a)) and centrifuged at a high speed for five minutes. The tissue, along with the serum and the small amount of blood, becomes tightly packed in the bottom of the tube, (Fig. I (b)).

2. The water is removed as completely as possible with a capillary pipette and replaced with neutral formaldehyde solution.

3. With a platinum loop the tissue mass is carefully loosened from the tube so that it is completely surrounded by the solution and allowed to fix for twelve to twenty-four hours. *The tissue mass must be loosened from the walls of the*

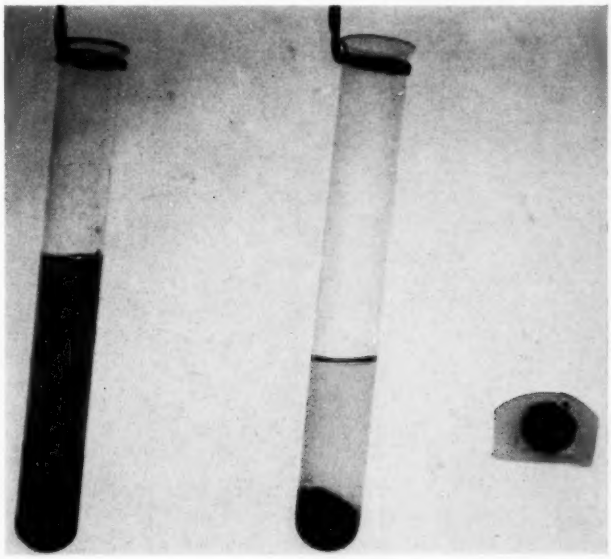

FIG. 1

tube before fixation or it will become so adherent that it will not come out as one piece.

4. When the formaldehyde is poured off the tissue mass has assumed a permanent shape in the form of a half sphere. With some care this mass will not break up and can be successively handled through all solutions. When the mass is blocked in paraffin, (Fig. I (c)) it is placed so that sections are cut from the flat side. The resulting sections are round and much more of the tissue can be studied because of the compactness of the pieces.

This method has been in use in my laboratory for the past year and curettings have become no more difficult to prepare than any other block of tissue.

SPIROCHETAL STAIN ON PARAFFIN SECTION*

THELMA GARVIN

From the Department of Pathology of the Nelson Morris Institute, Michael Reese Hospital, Chicago, Ill.

A workable stain for Spirochaeta pallida on paraffin sections is essential in every laboratory. The need for such a stain finally led to a modification of one of the methods which had been used with good results on frozen sections. This was a modification of the original Kanzler's stain.[1, 2, 3, 4]

In the following this method is briefly outlined, the effects of variation in time, temperature and concentration having been tried for each step.

1. Formalin fixed material embedded in paraffin, sectioned at 5 micra and mounted on ordinary slides with egg-albumin fixative.
2. Xylols, alcohol, distilled water.
3. Ammonium bromide formalin 2 hrs. or preferably over night: Ammonium bromide, 1 gram; formalin 40 per cent neutral, 7 cc.; distilled water, 48 cc.
4. Pyridine ½ to ¾ hour. Wash thoroughly in distilled water.
5. Uranium nitrate 0.5 per cent 20 min. Wash quickly in distilled water.
6. Silver nitrate 1.5 per cent 55–60°C. ¾ hour. Wash quickly in distilled water.
7. Transfer to modified Hortega's solution for 20 seconds, moving slide (to 5 cc. of 10 per cent silver nitrate solution add 20 cc. of 2.5 per cent solution sodium carbonate. Add pure conc. ammonium hydroxide drop by drop until ppt. is just dissolved. Add 15 cc. of distilled water).
8. Transfer without washing to a solution of 5 cc. of 40 per cent neutral formalin diluted to 100 cc. with distilled water, moving constantly, until solutions are yellow to yellow brown, 5–10 secs.
9. Wash in several changes of distilled water.
10. Alcohols, xylols and mount in balsam.

The organisms stand out dark brown to black against a yellow to light brown background (fig. 1.).

Chemically pure reagents should be used throughout and time, temperature and concentration closely adhered to.

If sections are left too long in uranium solution, the organisms

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take up the uranium nitrate and do not stain deeply with the silver. Too long a time in the silver solution causes the tissue to stain darkly thereby obscuring the organisms. Rapid movement of the slide in the modified Hortega's solution and the formalin developer prevents a precipitate of silver from forming on the sections.

This method has given consistently good results and has supplied the need for a good spirochete stain which can be applied

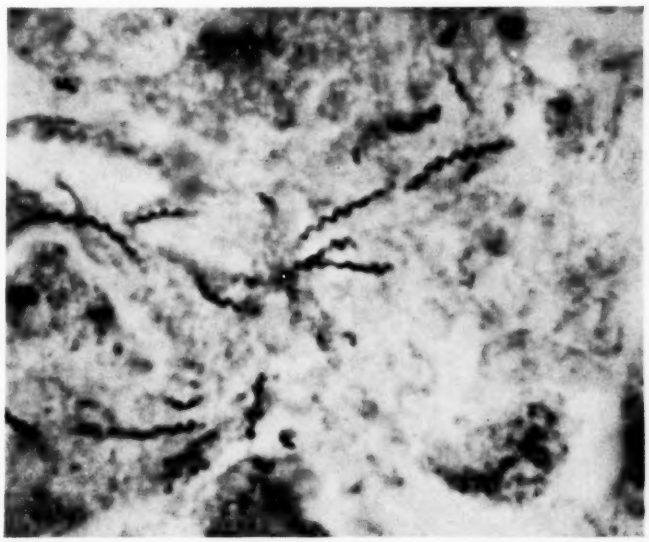

FIG. 1. SPIROCHAETA PALLIDA IN CONGENITAL SYPHILIS OF THE LIVER. ×2025
MODIFIED KANZLER STAIN

to tissues, limited in quantity, that have already been embedded in paraffin, and to those tissues which can not be handled easily as frozen sections.

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AN IMPROVED STAIN TECHNIC FOR OPSONO-PHAGOCYTIC TESTING IN BRUCELLOSIS

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It would seem that thin film staining in the Opsono-phagocytic tests in the diagnosis of Brucellosis is not without certain difficulties in interpretation. It would appear that some of these difficulties may be solved through the applications of thick smear staining. In thick smears, the erthyrocytes are not stained, and, if a non-granulophilic stain is used, the thick smear technic may even obviate the difficulties encountered in the Wright or Giemsa methods, wherein cytoplasmic granules might be mistaken for *Brucella*. The technic for thick smear staining in the Opsono-phagocytic test is as follows:

A simple 1 per cent Toluidine Blue solution is made in distilled water. Thick smears, unfixed, are allowed to remain in the solution for three minutes. They are then carefully washed with distilled water, allowed to dry, and examined under the oil immersion lens. The distilled water in the dye hemolyzes the erythrocytes; the toluidine blue slightly stains the white cells; the *Brucella* are very clearly stained a dark blue. The granules of the polymorphonuclears do not stain, and hence an accurate count of the number of organisms in a cell can be made. Moreover, it speeds up the test in that four or five polymorphonuclears can be found in one field. Twenty-five to 100 cells are counted on each slide and the number of Brucella inside each phagocyte is counted and tabulated: 0 as none; 1–20 as slight; 20–40 as moderate; and 40– or more as many.

The literature has been reviewed and no other reference to this type of stain was found, although it seems improbable that it has not been described before.

PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

A RAPID AND ECONOMICAL METHOD FOR STAINING ROUTINE TISSUE SECTIONS WITH HEMATOXYLIN AND EOSIN

A. A. KRAJIAN, Arch. Path. **25**: 376. 1938

The new principle consists in the application of eosin after dehydration of the section. The preparation of the eosin is as follows:

1. Dissolve 5 grams of water soluble eosin in 10 cc. of distilled water.
2. Precipitate by adding 10 cc. of glacial acetic acid. Mix with a glass rod.
3. Place in oven at 56°C. for 12 to 16 hours or until all the water has evaporated.
4. Dissolve the dried acid eosin in 10 cc. of absolute alcohol and 20 cc. of acetone stirring with a glass rod. Allow undissolved particles to settle.
5. Remove the clear portion with a pipette and add to it 1500 cc. of corbolxylene (1 part of pure phenol crystals and 3 parts neutral xylene). The name eosinol has been given to this mixture. It keeps indefinitely.

In using the method the sections are stained in hematoxylin, differentiated in acid alcohol and put in tap water until blue. They are then dehydrated in 95 per cent alcohol, the excess wiped off and dehydration completed by the addition of a few drops of absolute alcohol. Repeat the last step and counterstain in the eosinol (10 to 30 seconds depending on the strength of the solution), treat with carbolxylene 3 minutes, pass through three changes of xylene and mount in dammar dissolved in neutral histologic xylene (Eastman Kodak Co.).

The advantages claimed by the author are saving of alcohol and a more powerful and rapid stain.

PRESERVATION OF COMPLEMENT FOR THE WASSERMANN REACTION

C. A. GREEN: J. Path. & Bact. **46**: 382, 1938

Of the various methods described for the preservation of complement the author prefers that of Sonnenschein (Ztschr. f. Immunitatsforsch. u. exper. Therap. **67**: 512 1930) which is as follows:

Blood is obtained by severing the neck vessels of several guinea pigs, left overnight at 0°C. and the serum separated. To a given volume of the serum is added on equal volume of the following mixture.

Sodium acetate	12.0 grams
Boric acid	4.0 grams
Sterile distilled water to	100.0 cc.

The mixture is stored at 4°C. It is better to store in bottles containing a volume just in excess of the required for a day's tests. There is little or no loss of activity after several (7–12) month's storage.

A METHOD OF STAINING HAIR AND EPITHELIAL SCALES

D. A. BERHERIAN, Arch. Derm. & Syph. **36:** 1171, 1937

1. Cut scales in small pieces (1–2 mm.) place on slide and cover with 50 per cent glacial acetic acid in water and dry in incubator. This fixes the scales to the slide.

2. Defat, clear, and hydrate as follows: flood 2–3 times with ether allowing ether to remain on slide 20–30 seconds each time, flood slide consecutively with absolute, 95, 70, and 50 per cent alcohol. The alcohol is kept on 30–60 seconds each time.

3. Stain 3–5 minutes with Martinotti's toluidine blue.

Toluidine blue	1.0 gram
Lithium Carbonate	0.5 gram
Distilled water	75.0 cc.

After dye is completely dissolved add 20 cc. of glycerin and 5 cc. of 95 per cent alcohol.

4. Wash slide in water and differentiate in 0.5 per cent acetic acid.

5. Dehydrate in acetone, clear in xylene and mount in balsam or better euparol.

A NEW CHOCOLATE AGAR FOR CULTURE OF THE GONOCOCCUS

C. H. E. BECK, J. Lab. & Clin. Med. **23:** 415, 1938

1. Dissolve 77 grams of Bacto-North Gelatin Agar (Spray's formula) in 1000 cc. of distilled water by boiling several minutes.

2. Dispense in 1 liter Erlenmeyer flasks 500 cc. to each flask.

3. Stop flasks with gauze and cotton plugs, cap with heavy paper and autoclave at 20 pounds pressure for thirty to fifty minutes.

4. Cool to 55 to 60°C. add 50 cc. of citrated beef blood to each flask, rotate gently to insure thorough mixing.

5. Place flasks in a water bath at 60 to 65°C. and gradually raise the temperature to 100°C. Fifteen to twenty minutes should be consumed in doing this. Mix frequently by rotating the flask care being exercised to prevent foam. Hold at 100°C. for twenty minutes.

6. Cool rapidly to 55°C. and pour into Petri dishes.

The beef blood can be obtained at the slaughter house. Asepsis is not necessary since the heating process kills all ordinary contaminants. The plates keep well in the ice box. Satisfactory results are obtained after four to five weeks' storage.

Excellent growth is obtained in twenty four hours of even weakly growing strains.

ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

CLEANING COMPOUND FOR GLASSWARE

"For cleaning glassware, especially pipettes, cylinders, and flasks, hypochlorite solutions have the advantage of mechanical agitation by virtue of the property of hypochlorite to foam in the presence of organic matter.

We have used a commercial preparation marked under the name HILEX, made by the Hilex Co., St. Paul, Minn., which contains 5 per cent hypochlorite. It is obtainable at many grocery stores at approximately $1.00 a gallon.

It may be used in from 20 per cent to full strength—the latter is particularly useful in removing film from blood diluting pipettes. Thorough rinsing is, of course, essential."—FREDERICK H. LAMB, Davenport, Iowa.

DEHYDRATING AGENTS

In a comparative study of four dehydrating agents—cellosolve, tertiary butyl alcohol, dioxan, and acetone—on human tissues and following various fixatives, Magruder obtained the best results with dioxan and acetone. He prefers to fix in hot, concentrated commercial formalin for five minutes, wash in running water ten minutes, dehydrate in two changes of full strength dioxan or acetone one to one and a half hours, and embed directly in paraffin. Sections are mounted on slides with albumin fixative, thoroughly dried, passed through xylol to the dehydrating agent for a minute or more and then to distilled water. Stain in the usual way with Delafield's hematoxylin, destain if desired, wash, and pass into the dehydrating fluid. If acetone is used the eosin can be dissolved in it, thus counterstaining and dehydrating at the same time, but the sections must be cleared in xylol. In the case of dioxan stain with aqueous eosin, dehydrate in full strength reagent, and mount directly in balsam. S. R. MAGRUDER, J. Lab. & Clin. Med. **23:** 405, 1938.

GUM DAMMAR FOR MOUNTING SECTIONS

Evans claims the following advantages of gum dammar over balsam: the slides are much cleaner, they dry quicker, will not stick together if allowed to dry for a few months, they do not discolor, and do not fade. He prepares it thus. Dissolve the resin in "neutral histological (practical) xylene" specially prepared by the Eastman Kodak Company. Place the ingredients in a large stoppered bottle in the incubator and stirring with a glass rod several times daily until the mixture is of a heavy syrupy consistency (about a week.) Stain through four layers of clean gauze and store in a dark bottle. If the resultant solution is found too thin leave the unstoppered bottle in the incubator for evaporation until the proper consistency has been reached. NEWTON EVANS, Arch. Path. **25:** 83, 1938.

COUNTERSTAIN FOR THE GRAM METHOD

A recent article in one of the leading journals describes the use of Pappenheim's pyronin, methyl green mixture as a counterstain for the Gram method, giving the original formula. According to Conn (Biological Stains 3rd Ed. Geneva, N. Y., Commission on Standardization of Biological Stains, page 129, 1936) the American pyronins are so much more concentrated than those available before the war that a different formula is necessary. The following is recommended by him:

Methyl green (dye content 55–60 per cent)	1.0 gram
Pyronin B (certified product)	0.25 gram
Ethyl alcohol, 95 per cent	5.0 cc.
Glycerol	20.0 cc.
Phenol, 2 per cent aqueous sol.	100.0 cc.

The chief advantage of the stain is that it colors bacteria red while nuclei and nuclear fragments are stained blue or violet.